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N/C-4 substituted azetidin-2-ones: Synthesis and preliminary evaluation as new class of antimicrobial agents

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Abstract—A series of 3-chloro-4-(3-methoxy-4-acetyloxyphenyl)-1-[3-oxo-3-(phenylamino)propanamido] azetidin-2-ones **3a**–**g** and 3-chloro-4-[2-hydroxy-5-(nitro substituted phenylazo)phenyl]-1-phenylazetidin-2-ones **6a**–**h** were synthesized using appropriate synthetic route. Structures of all the synthesized compounds were established on the basis of elemental analysis and spectroscopic data. The antimicrobial activity of the synthesized compounds was screened against several microbes. Several of these molecules showed potent antimicrobial activity against *Bacillus anthracis*, *Staphyococcus aureus* and *Candida albicans* and significant structure–activity relationship (SAR) trends.

Nitrogen heterocycles are the basis of many essential pharmaceuticals and of many physiologically active natural products.[1] The 2-azetidinone (β-lactam) ring system is the common structural feature of a number of broad spectrum β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, nocardicins and monobactams, which have been widely used as chemotherapeutic agents to treat bacterial infections and microbial diseases.[2,3] These molecules operate by forming a covalent adduct with membrane-bound bacterial transpeptidases, which are also known as penicillin binding proteins (PBPs), involved in the biosynthesis of cell walls.[4,5] These mechanism-based inhibitors prevent the construction of cell wall and eventually lead to cell lysis and death. Moreover, due to their β-lactamase inhibitory action, 2-azetidinone-based heterocycles represent an attractive target of contemporary organic synthesis.[6] However, the efficacy of β-lactam antibiotics has been overshadowed in the last 20 years by the emergence of drug-resistant bacterial strains resulting from evolutionary responses to the widespread overuse and abuse of antibiotics in clinical traits.[7] Consequently, strategies to address this challenge lead to the design of improved versions of β-lactams with novel modes of action. It has been reported that introduction of different substituents to four-membered β-lactam nucleus tends to exert profound influence in conferring promising biological activities in these molecules.[8–10]

The recent discoveries of some 1,3,4-trisubstituted-β-lactams as new potent cholesterol absorption inhibitors,[11] human cytomegalovirus protease inhibitors[12] and thrombin inhibitors[13] justify a renewed interest in these compounds.[14,15] Furthermore, interest in the chemistry, synthesis and biology of the 2-azetidinone pharmacophore continues to be fuelled by their wide range of biological properties such as antibacterial,[16,17] antihyperglycemic,[18] anti-tumour,[19] anti-HIV,[20] anti-inflammatory and analgesic activities.[21] In addition, 2-azetidinones also display a broad range of enzyme-inhibitory activities.[22–26]

Looking to the promising antimicrobial activity of some 2-azetidinone[27,28] and 1,3-diketoamino[29] analogues synthesized earlier by us it was thought of interest to combine all the above-mentioned biolabile heterocyclic rings together in a molecular framework of hydrazones and imines in order to enhance the additive effect toward the biological activity. Keeping this in mind and in continuation of our earlier studies, we designed and synthesized 3-chloro-4-(3-methoxy-4-acetyloxyphenyl)-1-[3-oxo-3-(phenyl amino)propanamido] azetidin-2-ones **3a**–**g** and 3-chloro-4-[2-hydroxy-5-(nitro substituted phenylazo)phenyl]-1-phenylazetidin-2-ones **6a**–**h** with fascinating structural features. Moreover, in order to assess the antimicrobial potentiality of 2-azetidinone

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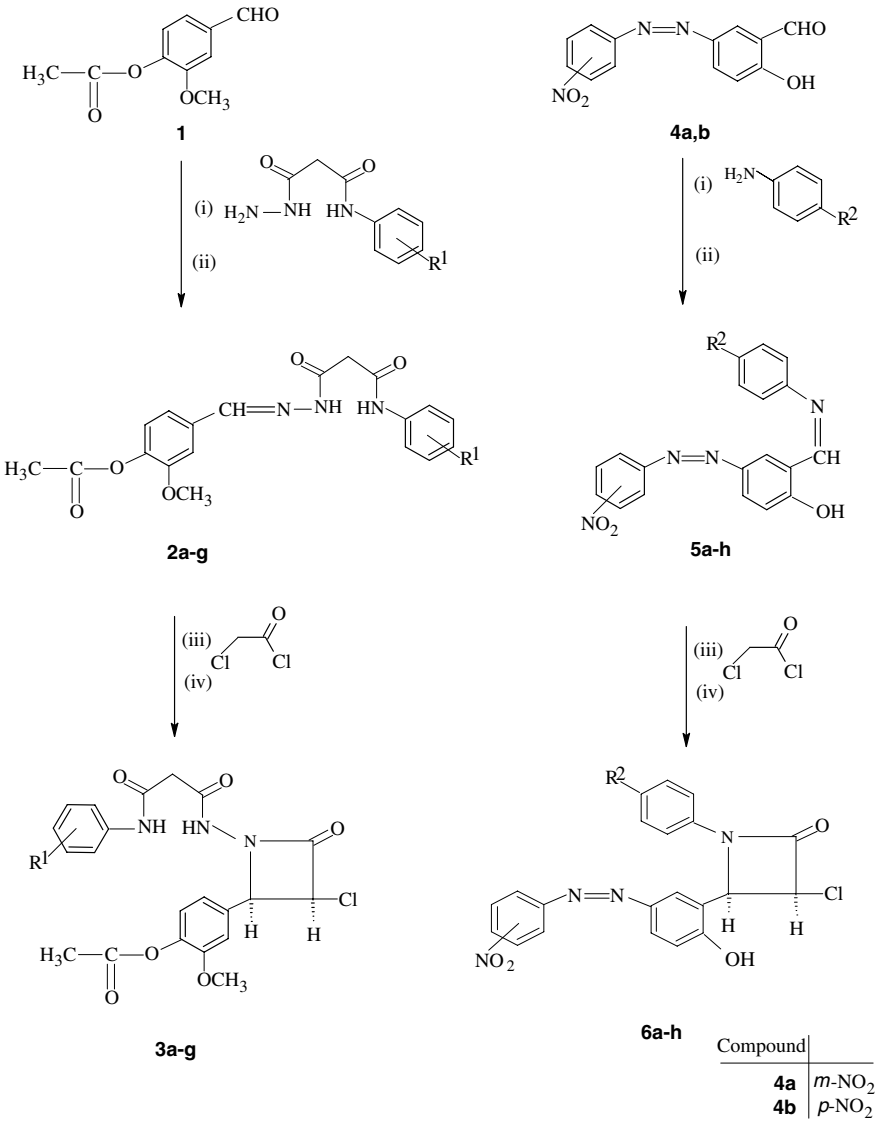
nucleus and to investigate the structure-activity-relationship, the constructed molecules were screened for their antibacterial and antifungal activities.

The synthetic pathway includes an electrocyclisation reaction of 1,3-diketoamino compounds **2a**–**g** and imine derivatives **5a**–**h** to give direct access to the desired 3-chloro-4-(3-methoxy-4-acetyloxyphenyl)-1-[3-oxo-3-(phenylamino)propanamido] azetidin-2-ones **3a**–**g** and 3-chloro-4-[2-hydroxy-5-(nitro substituted phenylazo)-phenyl]-1-phenylazetidin-2-ones **6a**–**h**, respectively (Scheme 1). The approach involves the initial preparation of precursors, viz.; 1-(3‴-methoxy-4‴-acetyloxybenzalhydrazino)-3-(phenylamino)propan-1,3-diones **2a**–**g** and substituted 2-hydroxy-5-(nitro substituted phenylazo) benzylidene anilines **5a**–**h**. Condensation of 3-methoxy-4-acetyloxy benzaldehyde **1** with substituted malonanilic acid hydrazides in ethanol yielded the 1,3-diketoamino analogs **2a**–**g**.[30] 3-methoxy-4-acetyloxy benzaldehyde **1** was prepared by the usual acetylation of 3-methoxy-4-hydroxy benzaldehyde at 0–5 °C. The substituted malonanilic acid hydrazides were synthesized by using reported methodology. The reactive imine derivatives **5a**–**h** were accessible via the reaction of an equimolar quantity of 2-hydroxy-5-(nitro substituted phenylazo) benzaldehyde **4a** and **b** and appropriate aromatic amines in ethanol at room temperature, which resulted in the formation of 2-hydroxy-5-(nitro substituted phenylazo) benzylidene anilines **5a**–**h** in excellent yield.[31] 2-Hydroxy-5-(nitrosubstituted phenylazo) benzaldehydes **4** were prepared by diazotization of 3-nitro aniline and 4-nitro aniline in acidic medium at 0–5 °C and coupling with salicylaldehyde in basic medium at 0–5 °C, respectively.

Further, the initially formed 1,3-diketoamino molecules **2a**–**g** and reactive imines **5a**–**h** underwent an electrocyclisation reaction of chloroacetyl chloride and triethyl amine in 1,4-dioxane afforded the target compounds 3-chloro-4-(3-methoxy-4-acetyloxyphenyl)-1-[3-oxo-3-(phenylamino)propanamido] azetidin-2-ones **3a**–**g** and 3-chloro-4-[2-hydroxy-5-(nitro substituted



Scheme 1. Reagents and conditions: (ii) EtOH, reflux, 2 h; (iv) Et_3N, 1,4-dioxane, stirred 1 h, reflux, 8 h.

phenylazo)phenyl]-1-phenylazetidin-2-ones **6a–h** in good yield.[32,33] Typically, ketenes are generated thermally from acid chlorides in presence of triethyl amine, which on subsequent reaction with reactive imines yield the desired *cis*-azetidin-2-ones.[34] Structures of newly obtained compounds have been ascertained on the basis of their consistent IR, ^{1}H NMR and mass spectral assignments.[35,36]

All the newly obtained azetidin-2-ones **3a–g** and **6a–h** were assayed in vitro for their growth inhibitory activity against pathogenic micro-organisms.The antibacterial activity was tested by the disc-diffusion method[37] using selected Gram positive and Gram negative strains of *Staphyococcus aureus, Bacillus anthracis* and *Salmonella typhi* and compared to reference drug—chloramphenicol. The experimental results of antibacterial activity indicated a variable degree of efficacy of the compounds against different strains of bacteria (Table 1). We next turned to examine the antifungal potentialities of azetidin-2-ones against pathogenic fungi viz.; *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans* by broth-dilution assay.[38] The minimum inhibitory concentration (MIC) values were determined by comparison with fluconazole as a reference standard and presented in Table 1.

The biological assay data have shown that screened azetidin-2-ones **3a–g** and **6a–h** exhibited moderate to potent activity against the tested microbes.

In Table 1, the most potent compounds are **3c**, **3d**, **3f**, **6b** and **6c**. Among the series of compounds the best growth inhibitory activity is of **3d** compound against *C. albicans*. Also **3c**, **3f**, **6b** and **6c** compounds have shown substantial activity against *C. albicans*. Furthermore compounds **3c**, **3d**, **3f**, **6a**, **6b** and **6c** have moderate inhibitory activity against *S. aureus*, *S. typhi* and *B. anthracis*. However, the growth inhibitory activities of compounds **3d**, **3f** and **6c** showed 2000 μ g/ml MIC value against *A. fumigatus*, **3c**, **3a**, **6g** showed 2000 μg/ml MIC value against *A. niger* and **6g** compound showed 250 μg/ml MIC value against *C. albicans* which is equal to standard drug fluconazole. On the contrary compound **3a**, **3b**, **3g**, **6d**, **6e**, **6f** and **6h** were found to be inactive against all the screened bacterial and fungal pathogens. The *m*-nitro substituted compounds **6a–d** showed better activity than compounds **6e–h** with nitro substituent present on para position.

The results of antimicrobial screening clearly indicate that the nature of substituents and their position on 2-azetidinone nucleus affected the in vitro activity significantly. All the three F, Cl and Br are classical bioisosteres so obey steric and electronic definition of classical bioisosteres. Chloro substituent frequently appears in many drugs and it follows the trend here also as chloro substituted compounds seem to be more potent than fluoro- and bromo-containing compounds hence the inhibitory activities of *p*-fluoro and *p*-chloro substituents are better than that of *p*-bromo substituent, suggesting that some steric hindrance might be present around the para position. Thus, *p*-chloro phenyl moiety and *p*-chloro phenyl 1,3-diketo aminopropane functionality on nitrogen of 2-azetidinone nucleus is an essential part of the pharmacophore.

Further, changing the position of nitro group from classical meta to ortho or para position of phenyl ring results in complete reduction of activity suggesting that the charge density is found to be greater at *o*, *p* than that of meta position. Therefore, *m*-nitrophenylazo moiety at C-4 of 2-azetidinone nucleus is also an essential part of the pharmacophore. Additionally, the azetidine-2-one derivatives (**3c**, **3d** and **3f**) substituted with the phenylcarbamoyl-acetylamino residue showed a more potent

Table 1. The in vitro antimicrobial activity of azetidin-2-ones **3a–g** and **6a–h**

Compound	R^1	R^2	Inhibition zone diameters[a]			MIC (μg/ml)[b]		
			S. aureus	*S. typhi*	*B. anthracis*	*A. fumigatus*	*A. niger*	*C. albicans*
3a	H	—	10	—	—	4000	4000	1000
3b	*o*-Cl	—	12	—	21	2000	1000	500
3c	*m*-Cl	—	26	17	22	2000	2000	125
3d	*p*-Cl	—	30	29	34	1000	1000	31.25
3e	*o*-NO_2	—	11	—	17	4000	4000	2000
3f	*m*-NO_2	—	27	31	33	1000	2000	62.5
3g	*p*-NO_2	—	—	—	12	4000	4000	1000
6a	*m*-NO_2	H	24	18	31	2000	4000	1000
6b	*m*-NO_2	*p*-F	29	21	28	1000	500	62.5
6c	*m*-NO_2	*p*-Cl	30	27	32	2000	1000	125
6d	*m*-NO_2	*p*-Br	11	08	17	4000	4000	—
6e	*p*-NO_2	H	08	—	10	—	4000	2000
6f	*p*-NO_2	*p*-F	—	—	12	4000	4000	2000
6g	*p*-NO_2	*p*-Cl	17	10	21	—	2000	250
6h	*p*-NO_2	*p*-Br	—	—	08	—	4000	4000
Chloromycetin	—	—	32	36	34	—	—	—
Fluconazole	—	—	—	—	—	2000	2000	250

Antibacterial susceptibility of compounds was measured in terms of zone of growth inhibitions.
(−) means no activity.
[a] Inhibition zone diameters in millimeters at 400 μg/ml concentration of compounds.
[b] The MIC value was defined as the lowest concentration of each chemical compound in the tubes (i.e., no turbidity) of inoculated fungi.

antimicrobial activity than the other derivatives **6a**–**h**. The chloro moiety of compound **3d** improved their antimicrobial activity significantly. In addition, we were able to identify some interesting structure–activity relationships around the phenyl moiety of phenylcarbamoylacetylamino residue that of phenyl azophenyl residue and *N*-phenyl residue on azetidinone ring. Our results should encourage the synthesis of N/C-4 substituted azetidine-2-one analogs to give new class of antimicrobial agents.

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30. Experimental: All melting points were measured in open capillary and are uncorrected.The chemicals and reagents used were of AR grade. The product mixtures were analyzed by thin-layer chromatography (TLC) on silica gel sheets. IR spectra (in cm^{-1}) were recorded on Perkin Elmer 337 spectrometer. ^1H NMR was recorded on a Varian EM-390 MHz NMR spectrometer in DMSO-d_6, chemical shifts are given in δ ppm using TMS as an internal standard. Mass spectral analysis was performed on Jeol SX-102 spectrometer using FAB technique. General procedure for the synthesis of 1-(3‴-methoxy-4‴-acetyloxy benzalhydrazino)-3-(phenylamino)propan-1,3-diones (**2a**–**g**): In a 250 mL round-bottomed flask, an equimolar solution (0.005 M) of compound **1** and substituted malonanilic acid hydrazides in EtOH was refluxed for 2 h at room temperature and left aside in an ice bath. The resulting precipitate was filtered, washed and recrystallised from petroleum ether (60–80°C).
31. General procedure for the synthesis of 2-hydroxy-5-(nitro substituted phenylazo) benzylidene anilines (**5a**–**h**): An equimolar mixture (0.005 M) of compound **4a,b** and appropriate anilines in ethanol was refluxed for 2 h. The reaction mixture was cooled in an ice bath and a drop of sulfuric acid was added to it. The product obtained was filtered, washed and recrystallised by EtOH as shiny reddish yellow crystals.
32. General procedure for the synthesis of 3-chloro-4-(3-methoxy-4-acetyloxyphenyl)-1-[3-oxo-3-(phenylamino)propanamido] azetidin-2-ones **3a**–**g**: In a closed vessel containing compound **2a**–**g** (0.001 M) in 20 mL of 1,4-dioxan, 0.095 mL of chloroacetyl chloride and 0.16 mL of triethylamine were added and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was then kept at room temperature for 30 min and further refluxed for 8 h. The filtrate was concentrated under reduced pressure and poured into ice-cold water. The product **3a**–**g** so obtained was recrystallised from methanol as light brown crystals.
33. General procedure for the synthesis of and 3-chloro-4-[2-hydroxy-5-(nitro substituted phenylazo)phenyl]-1-phenylazetidin-2-ones **6a**–**h**: In a closed vessel containing compound **4** (0.001 M) in 20 mL 1,4-dioxan, 0.095 mL of chloroacetyl chloride and 0.16 mL of triethylamine were added and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was then kept at room temperature for 30 min and further refluxed for 8 h. The filtrate was left for three days at room temperature and treated with ice-cold water. The coloured solid obtained was filtered, washed and recrystallised from 1:1 methanol + chloroform mixture.
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35. Analytical data of compound **3a**: mp (°C) 162–164, yield 68%: IR KBr (ν cm^{-1}) 3370 (N–H), 1770 (C═O, $OCOCH_3$), 1762 (C═O, four membered lactam), 1634,

2780 (C═O, 1,3-diketo), 1280, 1030 (C–O, OCH_3), ^1H NMR (δ) ppm 2.1 (s, 3H, $OCOCH_3$), 3.8 (s, 3H, OCH_3), 5.3 (d, 1H, $J_{3,4}$ = 4.4 Hz, C4-H), 5.9 (d, 1H, $J_{3,4}$ = 4.4 Hz, C3-H), 6.9 (s, 1H, NH), 7.2 (s, 1H, NH),7.4–7.7 (m, 8H, ArH). MS: Elemental analysis, found (calcd) (%) C, 55.04 (56.56): H, 4.19 (4.48); N, 9.03 (9.42).

36. Analytical data of compound **6a**: mp (°C) 188–190, yield 72%: IR KBr (ν cm^{-1}) 3512 (O–H), 1760 (C═O, four-membered lactam), 1578 (N═N), 1498 (N═O, asym), 1349 (N═O, sym), 1602, 1599, 1464 (C–C, ring str), ^1H NMR (δ) ppm 4.2 (s, 1H, OH), 5.1 (d, 1H, $J_{3,4}$ = 4.4 Hz, C4–H), 5.8 (d, 1H, $J_{3,4}$ = 4.4 Hz, C3–H), 7.1–7.4 (m, 12H, ArH), MS: Elemental analysis, found (calcd) (%) C, 61.23 (61.99): H, 3.18 (3.69); N, 13.41 (13.77).
37. In disc-diffusion assay, few colonies of organisms were inoculated in 2–5 mL nutrient broth and grown for 2.5 h. The agar plates were dried and inoculated by spreading the bacterial suspension evenly over it. The sterile paper discs (6 mm) impregnated with fixed dose viz., 400 μg/mL of compound were placed on the preinoculated surface. The disc-bearing plates were incubated at 37 °C and examined at 48 h for zone of inhibition, if any, around the disc. Chloromycetin was used in assay as a standard control drug. An additional negative control disc without any sample but impregnated with equivalent amount of solvent (DMF) was also used in the assay. The diameter of inhibition zone is directly proportional to the degree of sensitivity of bacterial strain and the concentration of compound under test.
38. In broth dilution assay, different concentrations of compound in the range from 15.62 to 8000 μg/mL in DMF were prepared using Sabouraud's Dextrose media in sterile test tubes and suspension of fungal cultures was inoculated in them. These tube dilutions were incubated at 25 °C for 72 h along with control. Fluconazole was also screened under similar conditions as reference drug. The dilution of a compound showing no visible growth of fungi was taken as minimum inhibitory concentration (MIC) of compound.